1	Λ
r	٦

HS_FDE6C	746 <mark>WEQ</mark>	GDLE	RTVL	QQQP	IPMMDRN	KRDELPKLO	2 V G F I D F V C T 785
Mm_PDE6C	WEQ	GDLE	RTVL	Q Q Q P	I PMMDR S	KKDELPKL	QVGFIDFVCT
<i>Gg-PDE6C</i>	WEQ	GDLE	RTVL	Q Q Q P	I PMMDR N	KGDELPKL	QVGFIDFVCT
<i>Rp_PDE6C</i>	WEQ	GDLE	RTVL	Q Q Q P	I PMMDRN	K G E E L P K L (QVGFIDFVCT
Dr_PDE6C	WEQ	GDLE	RTVL	DQQ P	IPMMDRN	KSDELPK MC	QCGFIDFVCS
Hs_PDE6A	WEQ	GDLE	RTVL	Q Q N P	I PMMDRN	KADELPKL	QVGFIDFVCT
Mm_PDE6A	WEQ	GDLE	RTVL	Q Q N P	I PMMDR <mark>N</mark>	KADELPKL	QVGFIDFVCT
<i>Rp_PDE6A</i>	WEQ	GDLE	RLVF	DENP	I PMMDR <mark>k</mark>	KADELPKL	QCGFIDFVCT
Dr_PDE6A	WEQ	GDLE	RTVL	EQQP	IPMMDRN	KAEELPKL	QCGFIDFVCS
Hs_PDE6B	WEQ	GDLE	RTVL	DQQ P	I PMMDRN	KAAELPKL	QVGFIDFVCT
Mm_PDE6B	WEQ	GDLE	RTVL	DQQ P	IPMMDRN	KAAELPKL	QVGFIDFVCT
<i>Gg-PDE6B</i>	WEQ	GDLE	ISVL	QQQP	I PMMDR R	KAAELPKL	QVGFIDFVCT
Rp_PDE6B	WEQ	GDLE	RTVL	QQQP	I PMMDRN	KSAELPKP	QCGFIDFVCT
Dr_PDE6B	WEQ	GDLE	RTVL	EQQP	IPMMDRN	KAAELPKLO	QCGFIDFVCT
Dr_PDE6B Pm_PDE6	WEQ WEQ	GDLE	R T V L R T V L	E <mark>Q Q P</mark> Q <mark>Q N P</mark>	I PMMDRN I PMMDRN	KAAELPKLO Kavelpklo	Q C G F I D F V C T Q C G F I D F V C T
Dr_PDE6B Pm_PDE6	WEQ WEQ	GDLE GDLE ∳	RTVL RTVL ♠	EQQP QQNP	IPMMDRN IPMMDRN ↑↑ ↑	KAAELPKLO KAVELPKLO	QCGFIDFVCT QC <mark>GFIDFVCT</mark> A
Dr_PDE6B Pm_PDE6 B	WEQ WEQ ♠	GDLE GDLE ∳	RTVL RTVL ∳	EQQP QQNP	IPMMDRN IPMMDRN ♠♠ ♠	KAAELPKLO Kav <mark>elpklo</mark>	QCGFIDFVCT QC <mark>GFIDFVCT</mark> A
Dr_PDE6B Pm_PDE6 B							
Dr_PDE6B Pm_PDE6 B Hs_PDE5	WEQ WEQ ↑	GDLE GDLE A GDRE	RTVL RTVL		I PMMDRN I PMMDRN ↑↑↑↑ TDLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO	CGFIDFVCT CGFIDFVCT A VGFIDAICL 826
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5	WEQ WEQ ↑ 787 FDQ FDQ	GDLE GDLE A GDRE GDRE	RTVL RTVL A RKEL RKEL		I PMMDRN I PMMDRN AAAA TDLMNRE ADLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO	CGFIDFVCT CGFIDFVCT A VGFIDAICL 826 VGFIDAICL
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5 0~ DDE5	WEQ WEQ ↑ 787 FDQ FDQ FDQ	GDLE GDLE GDRE GDRE GDRE	RTVL RTVL A RKEL RKEL RKEL	EQQP QQNP A NIEP NIEP	I PMMDRN I PMMDRN A A DLMNRE A DLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO KKNKIPSMO KKNKIPSMO	CGFIDFVCT CGFIDFVCT
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5 Gg_PDE5 St_PDE5	WEQ WEQ ↑ 787 FDQ FDQ FDQ FDQ	GDLE GDLE GDRE GDRE GDRE GDRE	RTVL RTVL RKEL RKEL RKEL RKEL	EQQP QQNP A NIEP NIEP NMEP	I PMMDRN I PMMDRN A A DLMNRE A DLMNRE T DLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO KKNKIPSMO KKNKIPSMO	CGFIDFVCT CGFIDFVCT
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5 Gg_PDE5 Xt_PDE5 Dr_PDE5	WEQ WEQ 787 FDQ FDQ FDQ FDQ YDQ	GDLE GDLE GDRE GDRE GDRE GDRE GDKE	RTVL RTVL RKEL RKEL RKEL RKEL RKEL	EQQP QQNP A NIEP NIEP NIEP	I PMMDRN I PMMDRN AAAA T DLMNRE ADLMNRE T DLMNRE I DLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO KKNKIPSMO KKNKIPSMO KKNKIPSMO	CGFIDFVCT CGFIDFVCT
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5 Gg_PDE5 Xt_PDE5 Dr_PDE5 Tr_PDE5	WEQ WEQ A 787 FDQ FDQ FDQ FDQ FDQ FEQ	GDLE GDLE GDRE GDRE GDRE GDRE GDKE GDKE	RTVL RTVL RKEL RKEL RKEL RKEL RREL RREL	EQQP QQNP A NIEP NIEP NIEP NIEP	I PMMDRN I PMMDRN ♦ ↑ ↑ T DLMNRE A DLMNRE A DLMNRE I DLMNRE I DLMNRE	KAAELPKLO KAVELPKLO KKNKIPSMO KKNKIPSMO KKNKIPSMO KKNKIPSMO KKDKIPSMO	CGFIDFVCT CGFIDFVCT VGFIDAICL SVGFIDAICL SVGFIDAICL SVGFIDAVCL SVGFIDAICT SVGFIDAICT
Dr_PDE6B Pm_PDE6 B Hs_PDE5 Bt_PDE5 Mm_PDE5 Gg_PDE5 Xt_PDE5 Dr_PDE5 Tn_PDE5	WEQ WEQ FDQ FDQ FDQ FDQ FDQ FDQ FCQ FEQ	GDLE GDLE GDRE GDRE GDRE GDKE GDKE GDKE	RTVL RTVL A RKEL RKEL RKEL RKEL RREL RREL	EQQP QQNP NIEP NIEP NIEP NIEP NIEP	I PMMDRN	KAAELPKLO KAVELPKLO KKNKIPSMO KKNKIPSMO KKNKIPSMO KKDKIPSMO KQDKIPSMO KKDKIPSMO	CGFIDFVCT CGFIDFVCT VGFIDAICL 2VGFIDAICL 2VGFIDAICL 2VGFIDAVCL 2VGFIDAICT 2VSFIDAICT

Figure S1. A. Sequence alignment of the M-loop/ α -helix-15 regions of PDE6C, PDE6A, and PDE6B from various species. Sequences corresponding to residues 746-785 of human cone PDE6C were used in the alignment. Arrows indicate PDE6-specific residues of PDE5/6cd contributing to the interaction with the P γ C-terminus and the H-M loop interface: Trp⁷⁸⁷, Leu⁷⁹², Val⁷⁹⁶, Gln⁷⁹⁹, Ile⁸⁰², Pro⁸⁰³, Met⁸⁰⁴, and Phe⁸²³ (using the PDE5 and PDE5/6cd numbering of residues). **B**. Sequence alignment of the M-loop/ α -helix-15 regions of PDE5 from vertebrate species. Arrows correspond to positions indicated in (**A**). *Hs* – *Homo sapiens, Bt* - *Bos taurus, Mm* - *Mus musculus, Gg* - *Gallus gallus, Rp* - *Rana pipiens, Xt* - *Xenopus tropicalis, Dr* - *Danio rerio, Tn* - *Tetraodon nigroviridis, Pm* - *Petromyzon marinus.*



Figure S2. The structure of the PDE10A2 D674A mutant catalytic domain in complex with cGMP (PDB ID: 20UU, chain A; wheat) was structurally aligned with the PDE5/6cd (PDB ID: 3JWR, chain A; *green*) bound with $P_{\gamma_{70-87}}$ (3JWR, chain C; *magenta*) using PyMOL 1.2r2. $P_{\gamma_{70-87}}$ is shown in surface representation and cGMP is shown in space-filling representation. In the superimposed model, $P_{\gamma_{70-87}}$ does not contact or clash with cGMP.

 Hs_PDE6C
 WEQGDLERTVLQQQPIPMMDRNKRDELPKLQVGFIDFVCT

 ancPDE6
 FEQGDLERKLLNQEPIPMMDRKKKDELPKMQVGFIDSVCT

 ancPDE5/6/11
 FEQGDLERKLNQEPIPMMDRKKKDELPKMQVGFIDSVCT

 Nv_PDE5/6
 FDQGDLEKEKLNAEPIPMMDRKKKNELPSMQVGFIDFICL

 Hm_PDE5/6
 FEQGDQERSKLSAEPIPMMDRGKHRDLPKMQVGFIDFVCM

Figure S3. Sequence alignment of the M-loop/ α -helix-15 regions of human Hs PDE6C (P51160), ancestral PDE6, ancestral PDE5/6/11, and PDE5/6-like PDEs from Nematostella vectensis (Nv PDE5/6, XP 001634744.1) and Hydra magnipapillata (Hm PDE5/6, XP 002161774). The sequences of the catalytic domains of the ancestral PDE6 (ancPDE6) and ancestral PDE5/6/11 (ancPDE5/6/11) enzymes were reconstructed using the ANCESCON software package (Max-Planck Institute's Bioinformatics Toolkit) and the multiple sequence alignment (ClustalW) of the catalytic domains of the following PDEs: Homo sapiens PDE1A (P54750), PDE2A (O00410), PDE3A (Q14432), PDE4A (P27815), PDE5A (O76074), PDE6A (P16499), PDE6B (P35913), PDE6C (P51160), PDE7A (Q13946), PDE8A (O60658), PDE9A (O76083), PDE10A (Q9Y233), PDE11 (Q9HCR9); Gallus gallus PDE1 (XP 418850.2), Danio rerio PDE4 (XP_695374.3); Petromyzon marinus PDE6 (ABO28525.1); Ciona savignyi PDE6 (Gene ID ENSCSAVG 0000005480); Ciona intestinalis PDE5 (Gene ID ENSCING0000003569), PDE6 (SNAP CIONA 00000036352), PDE10 (XP 002121759.1); Strongylocentrotus purpuratus PDE11 (XP 78196); Caenorhabditis elegans PDE2 (NP 001022707.1), PDE3 (NP 871943); Nematostella vectensis PDE1 (XP 001629364.1), PDE2 (XP 001630707.1), PDE4 (XP 001638196.1), PDE5 (XP 001631585.1), PDE5/6 (XP 001634744.1), PDE10 (XP 001624188.1,) PDE11 (XP 0011635720.1); Hydra magnipapillata PDE2 (XP 002162064.1), PDE5 (XP 002162868), PDE5/6 (XP 002161774), PDE10 XP_002165749.1), PDE11 (XP_002165584.1). Arrows correspond to positions of residues of PDE5/6cd contributing to the interaction with the Py C-terminus and the H-M loop interface (as in Figure S1).